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Water ferns *Azolla* spp. (Azollaceae) as new host plants for the small China-mark moth, *Cataclysta lemnata* (Linnaeus, 1758) (Lepidoptera, Crambidae, Acentropinae)

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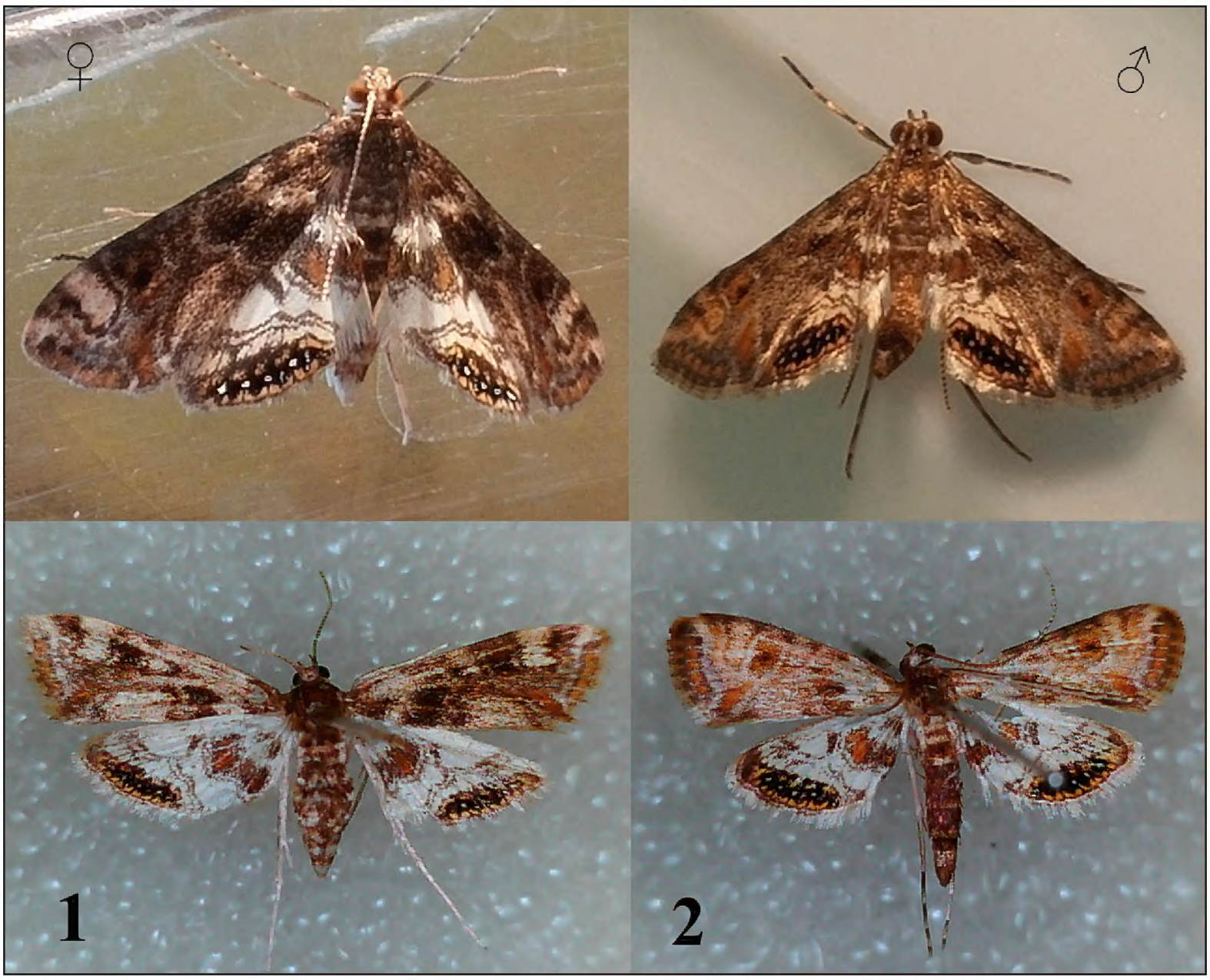
Abstract. Water ferns (*Azolla* spp., Azollaceae) are reported for the first time as host plants for the larvae of the small China-mark moth *Cataclysta lemnata* (Linnaeus) (Lepidoptera: Crambidae: Acentropinae) in rice fields and waterways of northern Iran. *Cataclysta lemnata* is a semi-aquatic species that has been recorded to feed on Lemnaceae and a few other aquatic plants. However, it has not been reported before on *Azolla* spp. Larvae use water fern as food source and shelter and, at high population density in the laboratory, they completely wiped water fern from the water surface. Feeding was confirmed after rearing more than eight continual generations of *C. lemnata* on water fern in the laboratory. Adults obtained this way are darker and have darker fuscous markings in both sexes compared with specimens previously reported and the pattern remains unchanged after several generations.

Introduction

Cataclysta lemnata (Linnaeus) is a small semi-aquatic moth belonging to the Crambidae subfamily Acentropinae. Acentropinae, previously known as Nymphulinae, are distributed worldwide and reach their highest diversity in tropical regions of South East Asia/Malaysia and in the Neotropical Region (Mey and Speidel 2008). Until 2008, a total of 50 genera and 737 described species were assigned to this subfamily and 23 new species were described from Africa in 2012 (Mey and Speidel 2008; Agassiz 2012). Nuss et al. (2016) list 745 species in 75 genera. Most of the Acentropinae species have aquatic larvae and in some species, e.g. *Parapoynx stratiotata* (Linnaeus), the larvae have gills for breathing under water (Hasenfuss 1960).

The genus *Cataclysta* was described by Hübner, 1825 with *Phalaena* (*Geometra*) *lemnata* Linnaeus, as the type species. There are 18 described species assigned to this genus (Nuss et al. 2016) and the full synonymy of *C. lemnata* is given by Speidel (2005). *Cataclysta lemnata* is sexually dimorphic (Figs 1, 2). The morphological characteristics of each sex have been described by Speidel (1984), Goater (1986), and Speidel (2005).



Figures 1–2. *Cataclysta lemnata* adults. **1.** Female; **2.** Male (live specimens above and prepared specimens below).

Azolla Lamarck is a genus of aquatic ferns and small-leaved floating plants divided into two subgenera: *Euazolla,* containing five species, and *Rhizosperma* with two species (Raja et al. 2012). Different species of *Azolla* provide various benefits such as organic nitrogen source, soil improvement and nutrient availability, weed suppression, food source for livestock, chickens, ducks and fishes (Anonymous 1987; Ferentinos et al. 2002). However, some of the species, such as *Azolla filiculoides* Lamarck and *Azolla pinnata* R. Brown (e.g. Barreto et al. 2000) are considered as major weeds in South Africa, Europe, and New Zealand (Hill 2003; Bodle 2008; Sadeghi et al. 2013a).

Azolla filiculoides is native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and the Americas (Costa et al. 2009), while *A. pinnata* is native to a large area of the tropics, subtropics, and warm temperate regions of Africa, Asia, and Australia (Sweet and Hills 1971; PIER 2007; Pemberton and Bodle 2009). Both species, and in particular *A. filiculoides*, are among the world's fastest growing aquatic macrophytes, with a doubling time in biomass of only 2–5 days (Zimmerman 1985; Taghi-Ganji et al. 2005).

In Iran, *A. filiculoides* is considered an alien species (JICA 2005; Delnavaz and Azimi 2009). Although the presence of another species is not yet confirmed, it seems that there is more than one *Azolla* species in some areas (Figs 3, 4). The Anzali and Amir-Kelayeh wetlands are two ecologi-

Figures 3–4. *Azolla* spp. in natural habitats that seem to represent different species. **3.** Different coloration of *Azolla* spp. in natural habitats; **4.** Different size and shape of each type.

cally important natural habitats in the north of Iran for breeding, wintering, and survival of many species of birds, fishes, and micro-organisms (Khoshechin 1993; Naddafi et al. 2005; Charkhabi and Sakizadeh 2006; Moradinasab et al. 2012). The invasion of *Azolla* into these wetlands (Delnavaz and Azimi 2009; Sadeghi et al. 2013a) is of concern because infestations reduce the quality of the water used for agriculture and humans, and simplify local aquatic food webs (Hill 1998). In addition, in rice fields with a poor drainage system this annoying weed could become problematic in transplanting rice-culturing systems because dense masses cause seedling death by covering the young seedlings and force the farmers to transplant anew.

Stenopelmus rufinasus Gyllenhal (Coleoptera: Curculionidae), a weevil native to the USA, has had a huge impact on *A. filiculoides* as a biological control agent in Africa, USA, and some other regions (Hill et al. 2008; Partt et al. 2013). However, due to the various positive and negative effects of other active herbivores on *Azolla* as biotic resistance factors and competitor species to the main biological agents, the identification of other active herbivores on *Azolla* was found to be desirable. Mechanical methods for control of water fern in Iran are impractical and there are no registered safe herbicides for aquatic ecosystems either. Therefore, control options for this obnoxious weed in Iran are limited. This situation stimulated the use of biological control as a sustainable strategy for the long-term management of *Azolla* spp. (Richerson and Grigarick 1967; McConnachie et al. 2003). We conducted surveys in rice fields over two years due to a lack of information on local herbivores attacking *Azolla* spp. in Iran, and the specific objective of this paper is to report the life history of *C. lemnata* attacking *Azolla* spp. in Iran.

Material and methods

Rearing: Laboratory colonies were established by collecting larvae and pupae from water fern collected in the Anzali (N 37°28'16", E 49°27'44") and Amir-Kelayeh (N37°20'35" E50°11'27") wetlands, waterways and experimental rice fields at the Rice Research Institute of Iran (RRII) (N 37°12'22.2", E 49°38'40.7", 80 m) from September to November 2014 and 2015.

Larvae and pupae were kept in transparent plastic containers (16.5 by 25 cm, diameter by height) filled with 1500 ml of water and 40 g of water fern. These containers were used for rearing in all tests. After adult emergence, the males and females were transferred to the mating containers. We used transparent plastic cups (8.5 by 11 cm, diameter by height) filled with 200 ml of water and 3 g of water fern, and covered with transparent cellophane for mating and we made small holes with a needle in the cellophane for ventilation. Two or three males with one female were released in each container to maximize the chances of fertilising eggs. Cups were changed daily until the female died. Water fern was also renewed regularly. Eggs were counted and kept in the incubator until larval emergence. First instar larvae released in rearing containers and all life stages were surveyed daily. Rearing containers were renewed every five days in order to prevent the growth of fungi and bacteria.

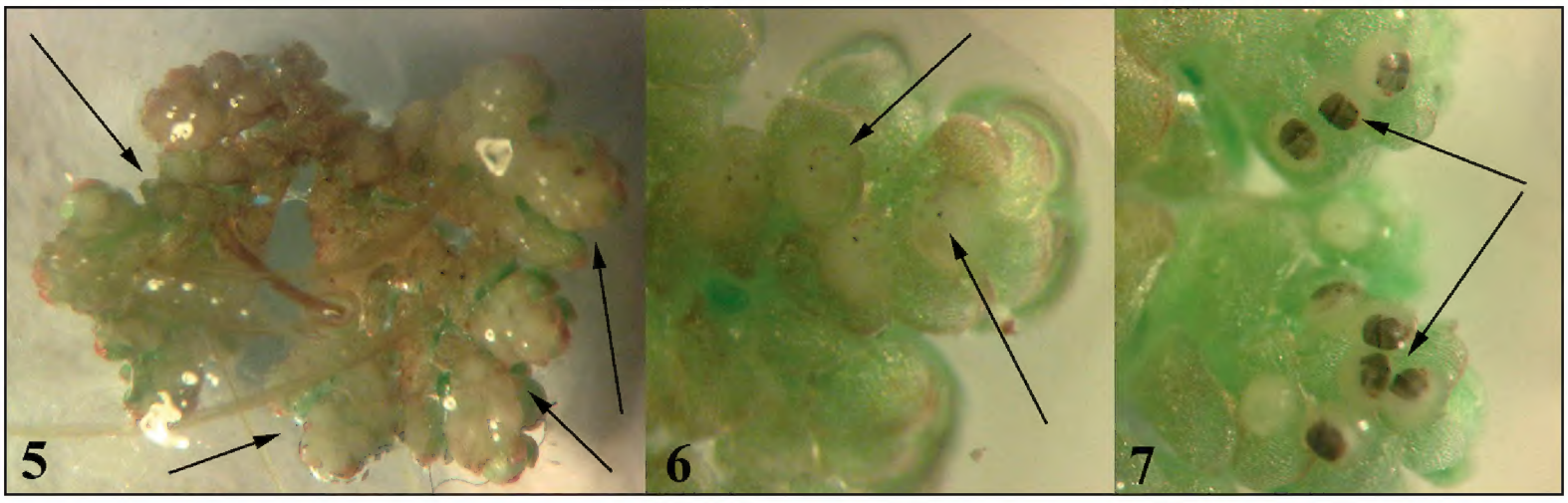
Identification: Forty adults were used for preliminary identification based on wing venation and other morphological characters. Dissection of both male and female genitalia (Figs 17–21) were made following the indications of Landry (2003) and Lee and Brown (2009). Dissections were conducted at the RRII laboratory and photographs sent to D. Agassiz in London for final determination.

Field observations: During 2014 and 2015, feeding damage observations by *C. lemnata* on water fern and other potential host plants were conducted in the waterways and experimental rice fields located at RRII.

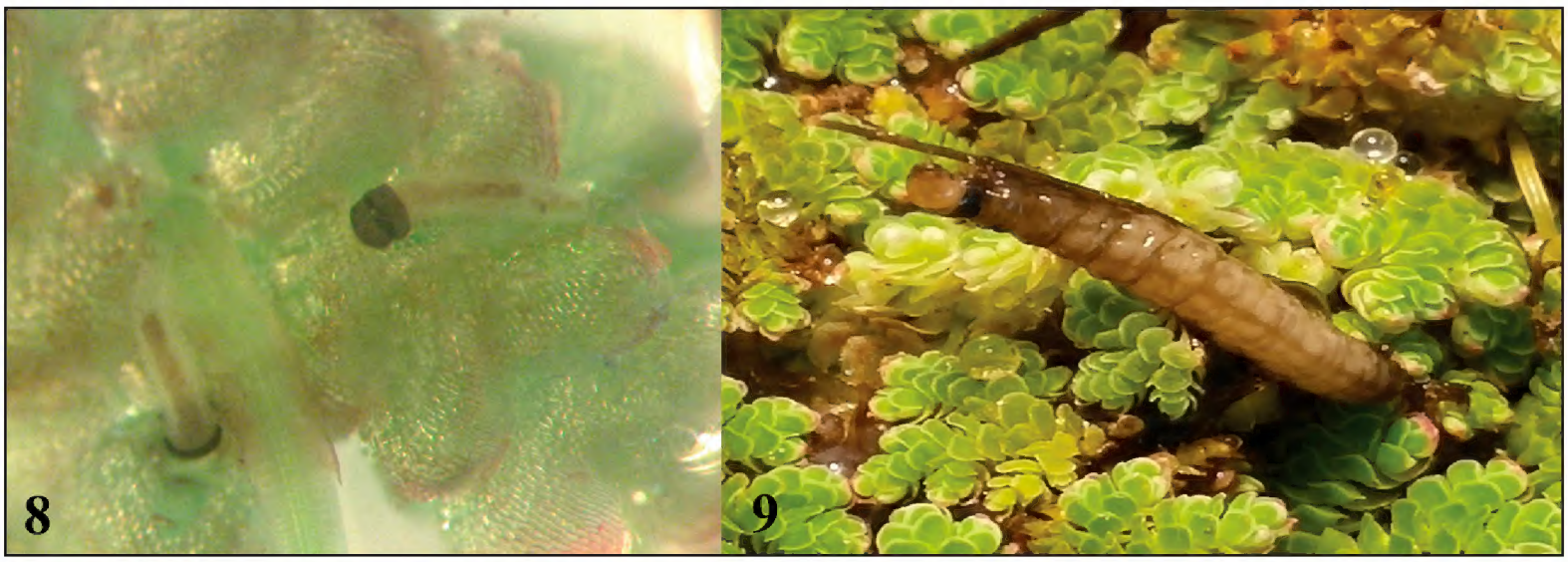
Results

Identification: Based on dissections of both male and female genitalia, the specimens were identified as *Cataclysta lemnata*.

Eggs: Less than 1 mm in diameter, globular, opaque to white (Fig. 5), with two darker spots on fertilised eggs after 24–48 hours (Fig. 6). These spots are larval eyes that become invisible as the



Figures 5–7. *Cataclysta lemnata* eggs on *Azolla*. **5.** A few hours after laying; **6.** After 48–72 hours; **7.** A few hours before hatching.



Figures 8–9. *Cataclysta lemnata* larvae on *Azolla*. **8.** First instar larva; **9.** Last instar larva.

head capsule gets darker. Before hatching, the head capsule becomes completely visible (Fig. 7). The eggs are laid singly or in groups of two or more under water on fern leaves. The developmental time of the eggs at 25–27°C was 5–7 days.

Larva: Newly hatched larvae are off-white, about 1.5 mm in length, with a black head capsule (Fig. 8). By the first moulting, after 3–5 days, the body colour changes to greenish grey or black and the head capsule becomes off-white. The last instar larva is dark grey to black and 15–18 mm in length with an off-white head capsule (Fig. 9). The developmental time of 100 larvae at 23–25°C was 25–47 days.

Pupa: Pupation usually occurs in shelters made from water fern leaves and a silky cover. As in other Acentropinae, the pupa of *C. lemnata* has chimney-like stigmata on abdominal segments 2 to 4. The pupa is reddish brown and approximately 7 mm in length (Figs 10–13). The development time of 50 pupae at 25–27°C was 7 days approximately.

Adults: The wingspan is 13–18 mm in male and 18–24 mm in female. Male with head and thorax light brown. Forewing off-white; costa fuscous, subbasal area with scattering of ochreous scales and dark brown patterns, discal spot with ochreous margin, median area with scattering of ochreous scales and antemedian ochreous spot, termen with a series of fuscous spots; fringe dirty yellow and brown alternately. Hindwing white with ochreous subbasal fascia, a scattering of pale fuscous scales, and one discal spot; termen with five conjoined black terminal spots, each with one

Figures 10–13. *Cataclysta lemnata* pupa. **10.** Pupa; **11.** Pupal shelter; **12.** Inside the pupal shelter with a silky layer; **13.** Pupa with chimney-like stigma.

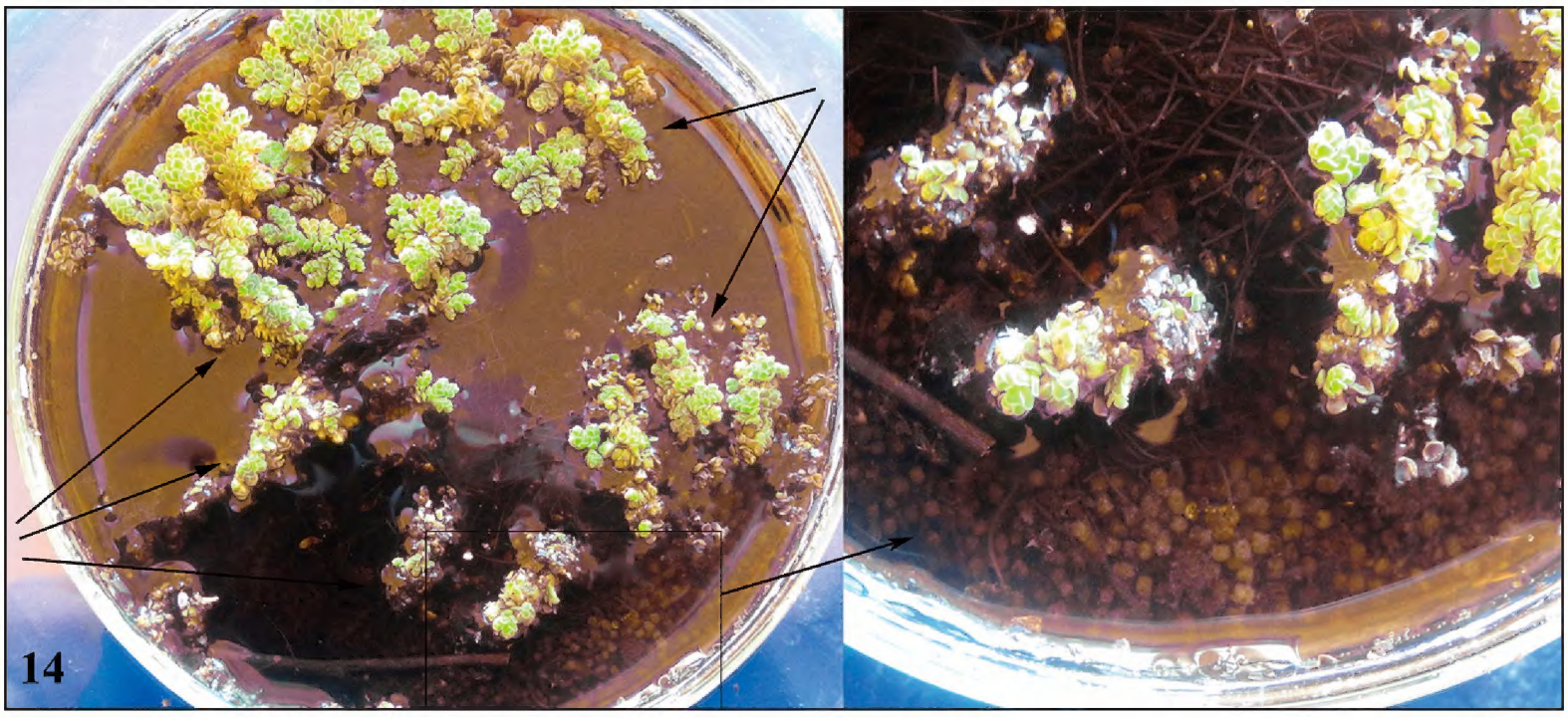


Figure 14. Evidence of *Cataclysta lemnata* activity. Larval shelters on surface; magnified: frass and stems at bottom of Petri dish.

silver dot; fringe white with dark line at base (Fig. 2). Female with head and thorax brown. Forewing fuscous and fringe with obscure markings as in males; hindwing as in male, with an irregular fuscous median fascia and a white lined fringe (Fig. 1).

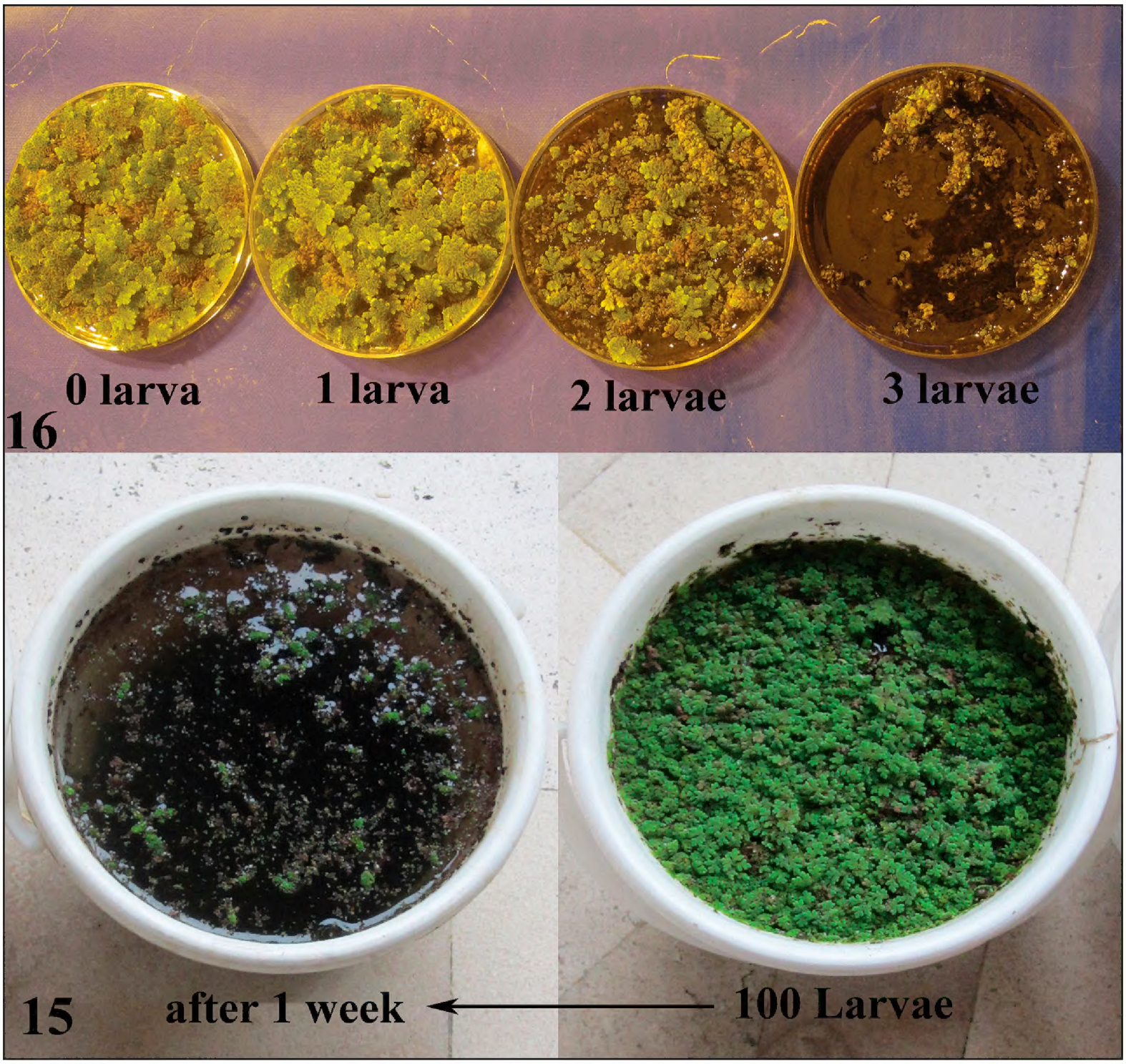
Evidence of damage on host plants: First instar larvae have been reported to be internal feeders on duckweed leaves (Chapman 1905; Wojtusiak and Wojtusiak 1960). However, on *Azolla* spp., first instar larvae feed on leaf buds, also internally. After a few days, they make shelters by attaching *Azolla* fronds to one another. The larvae remain in these shelters until pupation. They continue feeding and, as they develop, they make their shelters bigger. As the larvae get ready for pupation, they close the shelter with silk and adults emerge after almost a week. During heavy infestations in the laboratory, larvae almost wipe out water fern from the water surface and produce dregs made of cut stems and frass at the bottom of their containers (Fig. 14).

Discussion

The exotic aquatic fern, *A. filiculoides*, has invaded the Anzali Wetland. Since then, the overall ecological water quality, the habitat conditions, the numbers of migratory birds and fishes, and the aquatic plant diversity have declined in this ecosystem, particularly in the protected Selkeh wildlife refuge (Sadeghi et al. 2013a, b). In addition, the Amir-Kelayeh Wetland, which is another wildlife refuge, has become infested by this annoying weed as well (Farahpour et al. 2016). These changes are mainly related to anthropogenic activities (e.g. application of fertilizers, pesticides and herbicides in the paddy fields, and poor treatment of industrial waste in the watershed) and the invasion of *A. filiculoides* (JICA 2005; Ganjidoust et al. 2009; Sadeghi et al. 2013b). Moreover, although *Azolla* species are useful in paddy fields as an organic nitrogen source, for soil improvement, nutrient availability and weed suppression, they became problematic in fields with a poor drainage system in the northern region of Iran. At the same time, it seems that more than one species of *Azolla* invades aquatic ecosystems in Iran (Figs 3, 4) and this is another matter of concern. In order to manage *Azolla,* getting insight into biotic and abiotic factors affecting its growth in aquatic ecosystems of the north of Iran was necessary. Since 2011, some studies were conducted in the Anzali Wetland on some of the abiotic factors and the habitat requirements of *A. filiculoides* (Sadeghi et al. 2012a, b; 2013a, b). However, this weed's biotic resistance factors in Iran remained unclear.

During the present study, we investigated the activity of *C. lemnata* on *Azolla* spp. in rice fields and waterways. The first studies on *C. lemnata* suggested that this moth is a stenophagous species on Lemnaceae (Chapman 1905; Wojtusiak and Wojtusiak 1960) but it was reported later that this moth consumes various macrophytes (Vander Velde 1979, 1988; Pabis 2014). *Cataclysta lemnata* has been previously recorded from Iran (Speidel 2005), but *Azolla* spp. were never mentioned before as an element of the *C. lemnata* diet; in previous reports this species did not attack most other macrophytes beside Lemnaceae whilst the plants were fresh (Vander Velde 1979, 1988). In the rice fields and waterways of Iran, the moth is active from May until November. If the winter is mild, the larvae continue their activity slowly. They attack fresh water fern leaves and stems, but it seems that they prefer leaves to stems (Fig. 14). In the laboratory, larvae were capable of removing large parts of the floating cover of water ferns in Petri dish tests (Fig. 15) and when we increased the population density of larvae they almost wiped out the floating cover (Fig. 16).

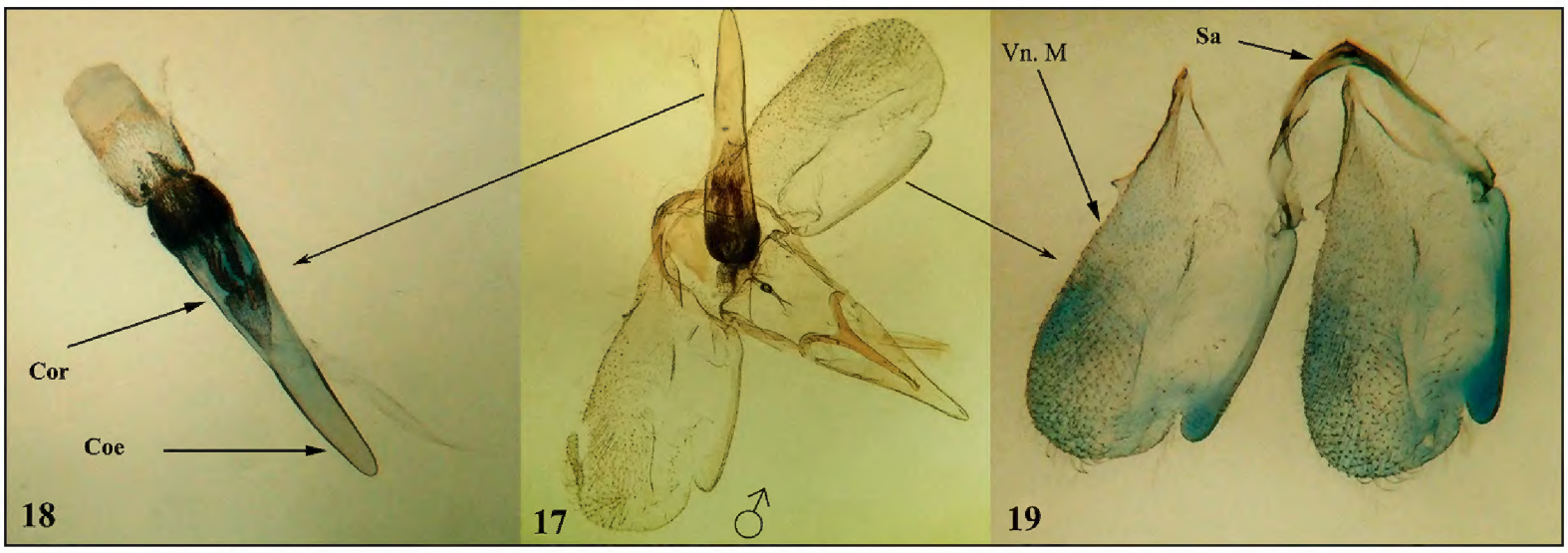
Speidel (1984, 2005) and Goater (1986) described and illustrated *C. lemnata* with a wingspan of 18–19 mm in the male and 22–24 mm in the female. The characteristics of the Iranian specimens are very similar to those mentioned in Speidel (1984, 2005) and Goater (1986), except that the Iranian specimens differ in the colouration of the head and thorax, the ochreous markings of the



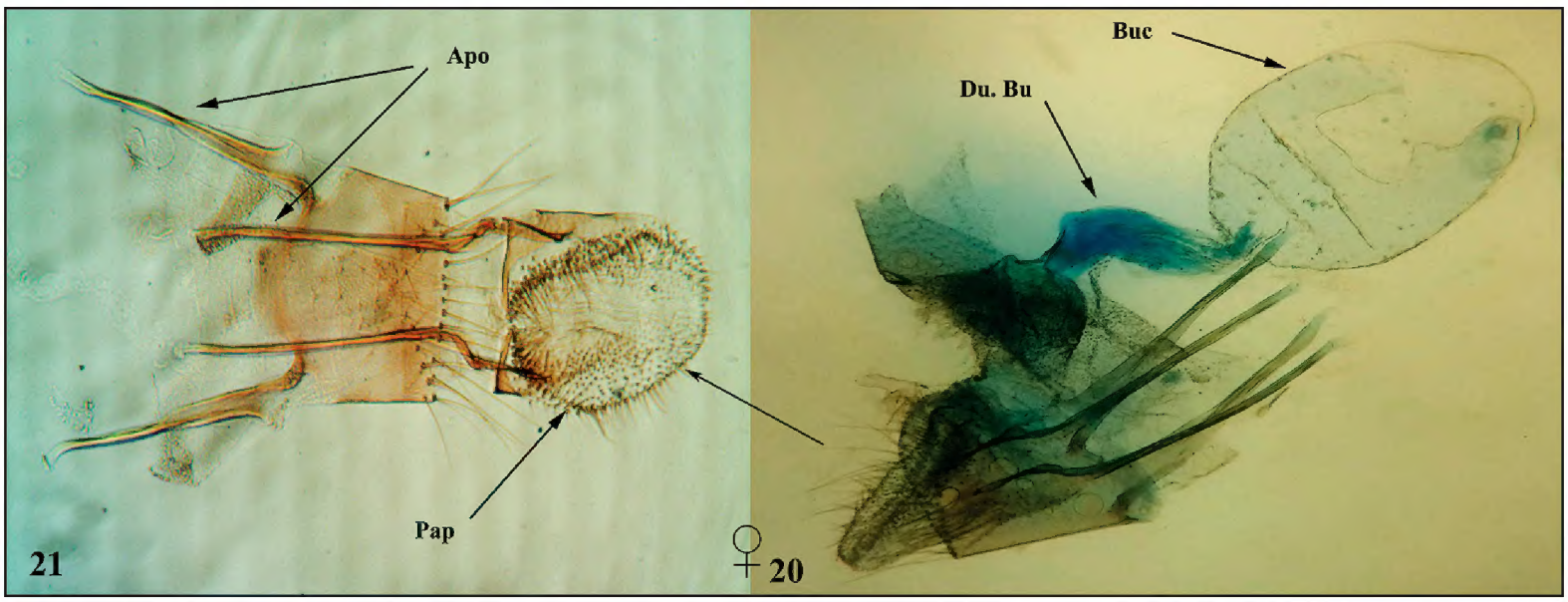
Figs 15–16. *Cataclysta lemnata* effects on water fern in the laboratory tests. **15.** Petri dish test with different population density; **16.** Larvae consuming the floating layer during a week of artificial infestation with high-density larval population in laboratory.

forewing, and the ochreous subbasal fascia of the hind wing. These differences are visible in both sexes, but they are more prominent in males.

The explanation for these external differences is unclear. Dissections of genitalia (Figs 17–21) showed that our specimens do not differ in that respect from those of other populations of *C. lemnata* occurring outside of Iran. It is possible that some of the differences among populations are due to phenotypic plasticity. There are many environmental conditions (e.g. temperature, diet, parasitism, etc.), that induce phenotypic plasticity in insects. For example, woolly bear caterpillars (*Grammia incorrupta* Edwards; Lepidoptera, Erebidae, Arctiinae) that were parasitized by *Exorista mella* Walker (Diptera, Tachinidae) ingest more of the plant toxins called pyrrolizidine alkaloids to confer increased resistance against parasitoids (Singer et al. 2009). *Chilo suppressalis* (Walker) (Crambidae, Crambinae) that feed on water-oat differ in adult body size and mating time compared with those that feed on



Figures 17–19. *Cataclysta lemnata* male genitalia slides. **17.** Ventral view with phallus; **18.** Phallus; **19.** Valva; **Cor** – cornuti; **Coe** – coecum; **Vn. M** – ventral margin; **Sa** – sacculus)



Figures 20–21. *Cataclysta lemnata* female genitalia. **20.** Lateral view; **21.** Last segments of the abdomen. **Buc** – bursa copulatrix; **Du. Bc** – ductus bursae; **Apo** – apophyses; **Pap** – papillae.

rice (Takasaki et al. 1969; Ishiguro et al. 2006). Perhaps the different colouration of *C. lemnata* feeding on *Azolla* spp. in Iran represents phenotypic plasticity related to a different diet. Nevertheless, we reared larvae on both *Azolla* spp. and *Lemna minor* as main host plants separately and imagines from both diets were phenotypically the same. Since this type of colouration is permanent among generations, it is possible that these specimens represent a new biotype of *C. lemnata* or perhaps even a new subspecies. However, we do not have any molecular or other evidence to test this hypothesis yet.

Our study is the first to record *Azolla* sp. as a host plant for *C. lemnata*. However, there are 12 other recorded Pyraloidea that feed on *Azolla* spp.: *Elophila enixalis* Swinhoe, *E. nigralbalis* Caradja, *E. responsalis* Walker, *E. turbata* Butler, *E. melagynalis* Agassiz, *E. manilensis* Hampson (Mochida et al. 1985, 1987), *E. africalis* Hampson (Roberts et al. 1998) and *Synclita obliteralis* Walker (Habeck 1991) in Acentropinae, *Ephestiopsis vishnu* Roesler and Kuppers (Mochida et al. 1985, 1987) and *Cryptoblabes gnidiella* Millière (Sasmal and Kulshreshtha 1984) in Phycitinae, and *Samea multiplicalis* Guenée (Knopf 1976) and *Diasemiopsis ramburialis* Duponchel (Farahpour et al. 2016) in Spilomelinae.

Although we briefly studied the biology of *C. lemnata* as a probable biotic resistance factor for water fern, many other important biological aspects such as population growth parameters and host preference in the natural habitat remain unclear. We could not find any specific parasitoid or predator for this moth although there are several generalist predators and parasitoids active in the rice fields (Shepard et al. 1987; Ooi and Shepard 1994) that could affect *C. lemnata* populations. However, some of the imagines in our colonies had a wing growth abnormality that seems to be genetic in origin. These imagines have distorted wings and were unable to fly properly, but they could mate as well as other imagines and produce fertile eggs. We did not use statistical or molecular methods for this study, but the number of eggs produced by these imagines was lower than for normal imagines. This abnormality appeared after at least four to five continual generations in the laboratory and only one or two imagines showed this abnormality during a whole generation.

Conclusion

Many studies have mentioned *Azolla* spp. as a weed (Hill 2003; Bodle 2008; Delnavaz and Azimi 2009). These invasive aquatic ferns are a major concern for biologists and ecologists dealing with conservation and management of wetland ecosystems due to the threats they may pose to the rich original biological diversity. They can have important harmful and irreversible impacts on wetlands as they may change the local fauna and flora (Sax et al. 2005; Vander Zanden and Olden 2008). Over the last few years, this obnoxious weed has invaded many wetlands in northern Iran (JICA 2005; Delnavaz and Azimi 2009; sadeghi et al. 2012a, b; 2013a, b). Due to the invasiveness of *Azola* spp., many native macrophytes such as duckweed have been eradicated in these valuable habitats (Sadeghi et al. 2012a). *Cataclysta lemnata* is an aquatic moth that was originally reported to feed on duckweeds and a few of other macrophytes. However, probably because of the eradication of its main host plant in many aquatic ecosystems of the northern region of Iran, this moth attacks exotic *Azolla* spp. water ferns and chooses them as new host plants. The effect of this moth on water fern as a biotic resistance factor is not clear yet and despite a rich fauna of aquatic and semi-aquatic insects, other biotic resistance factors of water fern in Iran are still poorly known. We have started our surveys on water fern biotic resistance factors in the rice fields and wetlands in the north of Iran in 2013 and this is the second report of our results. More studies are under way to uncover important facts on the biotic resistance factors of this annoying weed in northern regions of Iran.

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